
Regulation of Plant-Incorporated Protectants by the US Environmental Protection Agency

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A plant-incorporated protectant (PIP) is a pesticidal substance produced by a gene that has been inserted into a plant through transgenesis. EPA does not regulate the plant; in contrast to other regulatory agencies, we regulate the gene and the gene product.

As pointed out by Roger Beachy¹, the definition of a pesticide has been around since 1947, when the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) was promulgated. In addition to language about destroying, repelling, and/or mitigating pests—“pests” are defined in the statute—it deals with plant regulators (not plant-growth regulators). Admittedly the category is broad, but then “pesticide” is difficult to define.

As far as I know, there is no inclination or intention to regulate plants expressing plant-growth regulators as pesticides, although, certainly, a policy statement to that effect might be helpful. Today, I am speaking specifically about plants that are disease- and/or pest-resistant.

Four statutes influence our authority to regulate PIPs (Figure 1) of which I will focus on FIFRA, the main pesticide law, and the Federal Food Drug and Cosmetic Act (FFDCA) that we share with our colleagues at the Food and Drug Administration (FDA) regarding residues and tolerances.

FIFRA is unusual in terms of environmental statutes in that it considers benefits as well as risks, which is crucial with compounds that are inherently more toxic—typical nematicides, insecticides, *etc.*, for example—but it can apply to PIPs in terms of environmental safety or benefits and even economic safety and benefits. This does not apply to the Food Drug and Cosmetic Act.

¹Pages 19–28.

- **Federal Insecticide, Fungicide and Rodenticide Act – (FIFRA) pesticides**
- **Federal Food Drug and Cosmetic Act – (FFDCA) food and feed safety**
- **Food Quality Protection Act - (FQPA) amends FIFRA and FFDCA; sensitive groups included in assessment**
- **Endangered Species Act - (ESA) any impact on threatened or endangered species**

Figure 1. EPA's regulatory role.

In terms of initiating the process of achieving deregulation, most typically applicants start with an experimental use permit (Figure 2) to generate field data. It is particularly noteworthy that we do not generally regulate field trials at less than ten acres. Trials in excess of 10 acres are likely to be under an APHIS permit or notification process. If you're clever about it, you can plant multiple 9-acre plots, depending on how you design them. It is about cumulative acreage, but addressing a specific question with each cumulative 9.9 acres is acceptable and is not regulated by EPA as long as different pest-crop combinations are being evaluated in the plots of less than 10 acres. The point is to assist in the development of data characterizing the product and/or its chemistry that will be needed to ultimately achieve registration; it is not about producing seed for commercial purposes and the data generated cannot be used for promotional purposes. Of course, we need to know the origin of the transgene, its DNA sequence and the deduced amino acid sequence. Where *Agrobacterium* has been used, a plasmid map will be necessary. Also protein-expression levels are needed, typically determined at multiple stages in the plant's growth as well as at multiple locations; this need results from oversight being defined through a law governing pesticides. The content of the pesticidal substance must be elucidated, which is true for all pesticides, whether it's a liquid, a PIP, or a microbial compound. In addition, for purposes of insect-resistance management, it's important to know the accumulation of the protein in a leaf at whatever stage. That's particularly true with corn and cotton, and then, of course, an analytical method is required to detect the pesticidal substance, which is particularly important in trade.

Each event has a unique OECD² identifier. Although we often look at things parochially, we must keep in mind that we are in a global economy. From a scientific standpoint, I understand why people often say, "We are familiar with *Bt* and it would be nice to just give it a pass from now on." On the other hand, the Chinese, Koreans, Japanese, Australians, Brazilians and others may not agree with that. Putting a new product on the market

²Organization for Economic Co-operation and Development.

- **Cumulative acreage ≥ 10 A (4 HA) terrestrial or ≥ 1 A aquatic per year per pest requires EPA approval**
- **Food / feed tolerance required (at any size)**
- **EUPs are all time limited and require reporting of results as well as any adverse events**
 - [6(a)2 of FIFRA]
- **Products of EUPs are not eligible for advertising or promotion, but can be marketed with a tolerance**
- **EUPs are for research purposes**

Figure 2. Experimental use permits.

needs to be done with some forethought prior to actual commercialization. Asynchrony in approval is an ongoing problem in international trade.

For the vast majority of our guideline studies, test substances are proteins, which are often generated in a yeast or *E. coli*-based fermentation system (Figure 3). It is then the responsibility of the applicant to demonstrate the equivalency of the protein produced in the model system to that produced in the plant. To assess human-health effects of PIPs, we run acute oral toxicity maximum hazard dose tests with animals. Admittedly unrealistic, they are run for short periods to show any potential toxicity. In some other countries, long-term feeding studies are the norm, to reveal chronic and sub chronic toxicity. We have this option available if the first-tier testing indicates that there might be an issue.

- **Proteinaceous test substances are often produced in microbial systems**
- **It is the responsibility of the registrant to ensure the test substance from the native source (*in planta*) and microbe are equivalent**
- **Mr, MALDI-TOF / MS, and glycosylation status are all parameters to examine**
- **Bioassays can also be informational in establishing equivalency**

Figure 3. Test substances.

Biochemical properties are assessed. If the protein is an enzyme or a toxin that binds something, that needs to be demonstrated. Importantly, we do a lot of hematology comparisons both for toxicity and for allergenicity, based on known toxins and known allergens. In addition, digestibility studies assess allergenicity as well. This raises another point: when choosing a gene for transgenic work, it's best not to use a shellfish or a tree nut as the source, as it will raise red flags. That's not necessarily an insurmountable issue, but it may be costly in time and money.

In terms of the environmental or eco-assessment, non-target effects are a chief concern, on vertebrates and invertebrates alike (Figure 4). Because we are dealing with pesticides, environmental fate is also an important issue. What happens to the pesticidal substance: does it bind to clay particles, does it dissipate or is it degraded? In addition, does the transgene in question move outside of the cropping system to an indigenous or even feral relative? If so, it is something that we would consider in terms of potential adverse effects on the environment. If a tolerance is in place—in other words if we approve the gene product for consumption in animal feed or human food—then crop-to-crop gene flow is not an issue for us, although it may be an issue for USDA-APHIS. We require that, once you get to the field-trial stage, tolerances should be in place.

- **Avian oral /dietary toxicity studies**
 - **Quail, acute /42-day poultry feeding**
- **Freshwater fish oral / dietary toxicity studies**
 - **Rainbow trout or sunfish acute / catfish feeding**
- **Freshwater invertebrate testing (*Daphnia*)**
- **Honey bee oral toxicity testing**
- **Non-target arthropod testing**
- **Wild mammal toxicity (acute oral for rat / mouse)**
- **Estuarine and marine animal testing ***
- **Non-target plant toxicity studies ***
- **Endangered species considerations→ exposure determination**
 - * Often waived or satisfied with alternative data citation**

Figure 4. Data required for ecological effects on non-target organisms.

Figure 4 also shows some instances where corners may be rounded off to save some money. Some are very likely to be applicable—avian oral toxicity for a protein, for example. Typically, the mammal test has already been done; for human food tolerance, that study suffices. Although all of these data requirements need to be fulfilled, not all of them need to be fulfilled with empirical data generation; some may be fulfilled with a waiver rationale. My attorneys warn me not to call it a “waiver” because that has specific legal connotations. But it's a rationale. It may be a couple of paragraphs, a paper from

the literature, or it may be based on an exposure argument showing that the PIP will not affect a target organism or other source of concern. There are ways to get around that. We have never had a PIP produced in a marine or estuarine environment, so we've never asked for a grass-shrimp test.

We don't put a lot of weight in the 42-day poultry-feeding study, although other countries require it. Many of our registrants submit that, in which case we review it, but good data are needed from several other tests prior to getting to that stage.

NAVIGATING EPA

EPA sets tolerances—maximum residue levels—for pesticides in or on food and feed products. A pesticide residue present in or on a food or feed product that is not covered by a tolerance, or an exemption from the requirement of a tolerance, means that the product is considered “adulterated” under the FFDCA and the FDA is responsible for enforcement. That is to be avoided.

Tolerances are set by considering data from acute oral toxicity tests, sequence comparisons to known toxins and allergens, *in vitro* digestibility and the source of the gene. We look at homology to known allergens; a good database is available at the University of Nebraska. It's important to do such searches prior to progressing significantly, *e.g.* when building the constructs to verify that, by chance, the protein of interest doesn't have a hit, *i.e.* a 35% or greater homology over an 80 amino acid stretch. If that is triggered then more testing will be called for.

Frequently, people call and take advantage of our guidance. It's best to do so early on and not while in the midst of field trials only then to discover a problem.

As mentioned, most data requirements are designed for proteins as the test substance. If an RNA-interference approach is possible, it is likely to save time and money. People are spraying double-stranded RNAs, and bacterial vectors are being used to move RNAs into the environment. Although they're not proteins, questions remain in terms of how bioinformatics may aid predictability of action toward non-target organisms. For example, do the RNAs quickly degrade after contact with the soil? A number of questions need data generation. Clearly, RNAi won't solve everything, but where its use is possible, it should be considered. In addition, when inclusion of inert ingredients is needed, *e.g.* antibiotic and herbicide-tolerance markers, it makes sense to choose those that have been previously approved—glucuronidase, NPTII, EPSPS, PAD, *etc.*—again to save time and money.

Approval of green fluorescent protein has not yet been requested. The first such request will incur the cost of the required assessment. People have told me that a viable alternative approach will be to fuse NPTII and GUS. On the other hand, the tolerance for each of those individually may not apply to the fusion protein because truncation will change the sequence. Little things like that can cost more time and more money and, again, it's wise to contact us early—which doesn't cost anything—and hopefully, we can be of some help.

Now, in terms of the “barriers” that EPA's Office of Pesticide Programs promotes, PRN 11-3 (Figure 5) denotes the third notice issued in 2011 that provided guidance—in strong terms—for provision of data for applying for pesticide registration under FIFRA section 3

and FFDCA sections 408 and 409. The agency has a history of requiring particular formatting, to which there are advantages. In terms of record keeping, it lends efficiency when things are put together similarly and we have to find a piece of information pertaining to one of the thousands of products that we register. Initially it can be intimidating. We produce a registration manual that provides basic information (Figure 6). However, if you've never been through the process before, it can be helpful to employ an experienced consultant for guidance. We can provide a list of those who have worked with us, but we don't recommend specific people. On the other hand, some people have gone through the process without a regulatory consultant. Ralph Scorza and colleagues at USDA-ARS wrote a chapter for a book³ that Alan McHughen and I edited in which he describes—without sugarcoating—his trials and tribulations in obtaining deregulation of pox-virus resistant plums. I think he's correct, particularly in terms of his criticism of formatting requirements, which can be onerous. On the other hand, he did make it through the process, demonstrating that it is doable. Dr. Scorza is a good resource, particularly for academics who don't have a regulatory staff.

- **Format for data submitted to EPA under FIFRA section 3 and FFDCA sections 408 and 409**
- **Data packages submitted to the Agency outside of this format will most likely be rejected (BPPD may never see them)**
- **This is where a consultant comes in handy!**
- **http://www.epa.gov/pesticides/PR_Notices/pr2011-3.pdf**

Figure 5. PRN 11-3 formatting.

The Pesticide Registration Improvement Act—not a policy of the agency—is a statute passed by Congress. It was put together by stakeholders, NGOs, and industry and agency representatives in 2004 (Figure 7). In its third iteration, it sets fee schedules. Companies with 500 or more employees have a particular fee schedule. For a university or USDA-ARS scientist, waivers apply. For example a PI at Texas A&M will likely be eligible for a 75 percent waiver in comparison with the company fee. If a large company applies for deregulation of a new active ingredient for first food use, the fee may be \$400,000 to \$500,000 upfront. Tables are available setting out fee schedules for different groups of

³Wozniak CA McHughen A (Eds) (2012) Regulation of Agricultural Biotechnology: The United States and Canada. New York: Springer.

pesticides, These tables also provide firm timelines. These do not define when a registration will be granted, but rather when a decision will be made, on the assumption that a full and complete data package was submitted on day one. Some registrants have expressed approval of this approach because it removes some of the uncertainty; the previous queue system was inefficient.

➤ **The Pesticide Registration Manual ("Blue Book")**

<http://www.epa.gov/pesticides/bluebook/>

- Chapter 20: Forms & How to Obtain Them
- Chapter 21: Submitting Applications, Contacting EPA
- Chapter 11: Tolerance Petitions
- Appendix A: Guidance Documents
- Appendix B: Examples of Registrant Documents
- Appendix C: Forms Overview Table
- Appendix D: Examples of Completed Forms

Figure 6. Where to begin.

➤ **Statute that directs EPA to collect fees to regulate and register pesticides**

➤ **Establishes a decision* Time Frame**

- Fee Category Determines Fee & Time Frame

➤ **Assumes the Application is Complete:**

- Administrative Documents
- Data Requirements are Addressed
- Fees are Paid, or
- 25% (or 50%) of Fees Paid if Requesting a Fee Waiver

* Not necessarily a registration!

Figure 7. Pesticide Registration Improvement Act (PRIA 3).

SUMMARY

Consultations can be very informal. It's best if the applicant can visit EPA, but, otherwise, the initial consultation may be achieved by telephone, possibly a conference call, or email (Figure 8). Even a pre-submission meeting will be treated as confidential. At no cost, meeting minutes are generated, laying out what was discussed, what was agreed upon, what is left open, *etc.*, which initiates the process. It is advisable to have consultations early on because they may lead to beneficial alterations, for example in terms of generation of less data or discovery of a waiver rationale. As mentioned, the formatting requirements are mandatory, and assistance of a consultant is recommended. However, absence of a consultant should be not considered as a deal breaker. Others have achieved deregulation, and EPA staff—regulatory specialists—are there to assist.

- **Early consultation before submission of application is encouraged**
- **“Pre-submission” meeting(s) - confidential**
- **Determination of applicable data requirements needed early on in process**
- **Formatting requirements are mandatory and a consultant is recommended for formal submissions to the Agency**

Figure 8. Summary—navigating EPA.

Several websites are available for guidance; they are continuously updated and improved (Figure 9). Kimberley Nesci or I may be contacted to set up a pre-submission meeting or to answer other questions.

- <http://www.epa.gov/pesticides/biopesticides/regtools/biotech-reg-prod.htm>
- http://www.epa.gov/scipoly/sap/meetings/2000/october/brad3_enviroassessment.pdf
- http://www.epa.gov/pesticides/biopesticides/reg_of_biotech/eparegofbiotech.htm
- <http://www.epa.gov/scipoly/sap/meetings/2009/022509meeting.htm>
- http://www.epa.gov/oppbppd1/biopesticides/pips/pip_list.htm
- EPA – Kimberly Nesci, Branch Chief, Microbial Pesticides
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 - Chris Wozniak, Biotechnology Special Assistant
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Figure 9. Sources of assistance.



CHRIS WOZNIAK received his training in plant pathology and life sciences at the University of Nebraska at Lincoln, where his research efforts focused on cell differentiation and morphogenesis in *Sorghum bicolor*. He worked in David Galbraith's laboratory at UNL, developing insect-resistant cotton and with Lowell Owens at the USDA-Agricultural Research Service, Beltsville, developing transformation protocols in sugarbeet. He then joined the Sugarbeet Research Unit of the USDA-Agricultural Research Service in Fargo, ND, where he worked on biological control of an insect pest.

After 18 years in plant-science research, he entered the world of regulatory science at the US Environmental Protection Agency Office of Pesticide Programs. He performed risk assessments of microbial and plant-based pesticides, particularly in the areas of human health and environmental consequences of gene flow.

For four years, Dr. Wozniak served as the national program leader for Food Biotechnology and Microbiology at the USDA's Cooperative States Research, Education and Extension Service. While at CSREES, he directed two competitive grant programs in the areas of microbial food safety and environmental risk assessment for products of biotechnology.

In 2008, he rejoined the EPA as a biotechnology special assistant in the Office of Pesticide Programs, focusing on issues of biotechnology policy, interagency coordination of biotech regulations, and environmental risk assessment of plant-incorporated protectants.